Evaluating Intermediate Wheatgrass Germplasm for Use in a Breeding Program

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Contents

Abstract

Cultivars of intermediate wheatgrass, *Agropyron intermedium* (Host.) Beauv., used in the United States and Canada, have a narrow genetic base. Four released cultivars were selected from PI 98568, and two others have PI 98568 in their pedigree. Introductions are being systematically screened at Mead, Nebr., to identify accessions for use in breeding programs to expand the germplasm base and to improve the productivity of intermediate wheatgrass.

Considerable variation was found for agronomic traits in two sets of introductions. In 1975, space-transplanted seedlings for 36 accessions from Iran failed to survive the dry summer. Seedlings of the check cultivar 'Slate' did survive, demonstrating stress tolerance. From 1976 to 1977, I evaluated 38 accessions from the USDA Plant Introduction Station, Pullman, Wash., in a replicated space-transplanted nursery. Most accessions were considerably inferior to Slate. However, PI 345586, PI 273732, PI 273733, and PI 315355 were equal or superior to Slate in first-year growth, as measured by plant width and height, and exceeded the second-year mean plant forage yield of Slate by 71, 80, 63, and 45 percent, respectively.

Differences among accessions were the major source of variation for all the evaluated traits in the 1976 to 1977 nursery and broad sense heritability estimates among accessions were also high (0.80 or larger). There was also considerable variation within the high yielding accessions for first-year growth and second-year forage yield. It should be possible to improve the productivity of intermediate wheatgrass by using both among and within accession selection. The continued reliance on intermediate wheatgrass cultivars developed from a single introduction appears unwarranted.

Additional index words:
Agropyron intermedium, Agropyron trichophorum,
pubescent wheatgrass, forage yield.

Evaluating Intermediate Wheatgrass Germplasm for Use in a Breeding Program[1]

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Intermediate wheatgrass, *Agropyron intermedium* (Host.) Beauv., which is native to parts of Europe and Asia, is a productive cross-pollinated forage grass that is adapted to the central and northern Great Plains and to the intermountain West of the United States and Canada. The intermediate wheatgrasses used in these areas have a restricted genetic base since seven of the eight cultivars that have been released were developed primarily from two introductions (Hanson 1972). 'Nebraska 50,' 'Ree,' 'Oahe,' and 'Greenar' were developed from PI 98568; 'Chief' was developed from Ree and a USSR introduction; 'Amur' was selected from PI 131532; 'Slate' is based on clones selected from Amur and Nebraska 50; and 'Tegmar,' a dwarf cultivar used primarily for erosion control, was selected from PI 109219.

Dewey (1978) and Mariam and Ross (1972) concluded that pubescent wheatgrass, formerly *A. trichophorum* (Link) Richt., and intermediate wheatgrass are part of the same species complex and should be treated as a single species. Terrell (1977) lists the glabrous and pubescent types of intermediate wheatgrass as *A. intermedium* var. *intermedium* (Host.) Beauv. and *A. intermedium* var. *trichophorum* (Link) Halac, respectively. The results of Dewey (1978) and Mariam and Ross (1972) indicate that the use of the botanical variety names is superfluous. For the purpose of this paper, both the glabrous and pubescent intermediate wheatgrasses will be considered to be *A. intermedium.* Three pubescent intermediate wheatgrass cultivars, 'Greenleaf,' 'Topar,' and 'Luna' have been released. Luna and Topar were selected from PI 106831 and PI 107330, respectively, whereas Greenleaf was selected from various seed lots. Including these cultivars as part of the species does broaden the germplasm base of intermediate wheatgrass in North America.

The potential gain that can be made in a breeding program depends upon the genetic variability available. A primary objective of my research with intermediate wheatgrass has been to screen systematically introductions to identify accessions that can be used in breeding programs to improve its yield potential and to expand its germplasm base. This paper reports on the two sets of introductions that I evaluated to date and on the potential for improving the productivity of intermediate wheatgrass by using plant introductions in a breeding program.

The two types of seed lots that are usually available to grass breeders for evaluating plant introductions are original seed collected by a plant explorer or seed increased at a plant introduction station from original seed. Because the numbers of accessions being increased at any time precludes increasing seed in isolation, accessions of cross-pollinated grasses are increased in the same nursery and produce open-pollinated seed.

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Materials and Methods

In 1975, I evaluated 49 intermediate wheatgrass accessions from the 1972 Iran collection of Dewey (1978). Original seed of each accession was started in a greenhouse in January 1975. Seedlings of 36 accessions were transplanted into a nursery at the Mead Field Laboratory, Mead, Nebr., on May 14 and 15. The plots were single rows spaced 1 m apart. Each row contained 20 plants of one accession with plants spaced 1 m apart. Rows of the check cultivar Slate were located on the borders and every 20th row within the nursery. Thirteen accessions were not transplanted because of either poor seedling vigor or because too few seedlings were available to make up a complete row. The accessions were not replicated. I discontinued evaluating this nursery in September 1975 for reasons that will be explained in the results.

In 1976, 38 additional accessions that were from the initial increase of original seed were obtained from the U.S. Department of Agriculture Western Regional Plant Introduction Station, Pullman, Wash. I started seedlings in a greenhouse in February. The seedlings were transplanted to the Mead Field Laboratory on May 27 and 28. Size and spacing of the evaluation plots were the same as in the 1975 nursery. The experimental design was a randomized block with two replications. Four introductions were not replicated because of insufficient numbers of plants. Rows of Slate were located on the borders and every 11th row within the nursery. The nonborder rows of Slate were used as check rows.

The 1976 nursery was fertilized with 112 kg/ha N and 38 kg/ha P in September 1976 and with 112 kg/ha N and 31.4 kg/ha P in April 1977. DCPA (dimethyl tetrachloroterephthalate) and 2,4-D[(2,4-dichlorophenoxy) acetic acid] were used for weed control in both 1976 and 1977.

The width and height of each plant in the 1976 nursery were measured in cm after the end of the growing season on November 11. The nursery was mowed in early March 1977 to remove 1976 growth before the start of the 1977 growing season. I rated the individual plants visually on a 1 (excellent) to 5 (poor) scale for forage and seed production on July 8, 1977. Forage was harvested on July 12 with a flail-type forage harvester with a cutting height of 10 cm. This date was postanthesis and before seed maturation for all accessions. Individual plants were harvested for yield for 12 accessions that had above-average forage ratings and for the Slate check rows. I sampled two plants in each of these rows for moisture content and oven-dried the forage samples to determine dry matter percentages. Forage yields were harvested on a row basis for the other 26 accessions. All plants did not survive field transplanting. To simplify comparisons among accessions, all results are expressed as individual plant means. I obtained mean plant yields for the accessions harvested on a row basis by dividing total forage yield of a row by the number of plants harvested in the row. Yields were expressed as field weights unadjusted for moisture percentage.

Plant means were analyzed using analyses of variance procedures for the replicated accessions in the 1976 nursery to obtain mean square estimates of the genetic variance among accessions ($\hat{\sigma}^2_g$) and the error variance ($\hat{\sigma}^2_e$).

In this analyses, the mean of all Slate plants in a replication were used as the Slate mean for that replication. The error variance from this analyses was used to compute the least significant difference (LSD) value. Individual plant values for 10 of the strains were used in a separate analysis of variance to obtain estimates of the variance among plants within plots ($\hat{\sigma}^2_w$). Strains included in this analysis were one Slate plot from each replication and all the accessions harvested for yield on an individual plant basis except for PI 173630 and PI 220497, which did not have at least 16 plants per plot. Sixteen plants from each plot were used in the analysis to have a balanced data set.

The ratios, $\hat{\sigma}^2_g/(\hat{\sigma}^2_e/r + \hat{\sigma}^2_g)$ and $\hat{\sigma}^2_g/(\hat{\sigma}^2_w/rw + \hat{\sigma}^2_e/r + \hat{\sigma}^2_g)$ where r = number of replications and w = number of plants/plot, were calculated using the mean squares and mean square components obtained from the analyses of variance for plot means and individual plant values, respectively. I used methods described by Gardner (1963) to obtain estimates of variance components. These ratios are the genetic variances among lines divided by the phenotypic variances of their means and were used as estimates of heritability in the broad sense among accessions.

The within-plot ($\hat{\sigma}^2_w$) and between plot ($\hat{\sigma}^2_e$) variance estimates from the analyses of variance for forage yield using individual plant values were used to obtain estimates of the variance of a treatment mean ($s^2_{\bar{x}}$) and the resulting LSD value that would be obtained with various combinations of replications and plants within a plot. The equation used was $s^2_{\bar{x}} = (\hat{\sigma}^2_w + w\hat{\sigma}^2_e)/rw = \hat{\sigma}^2_w/rw + w\hat{\sigma}^2_e/rw = \hat{\sigma}^2_w/rw + \hat{\sigma}^2_e/r$ (Snedecor and Cochran 1967). Combinations of r and w were substituted in the equation to obtain estimates of $s^2_{\bar{x}}$ which, in turn, were used to calculate LSD estimates.

Correlations of the traits evaluated with forage yield were determined using plot mean values.

Results and Discussion

The summer of 1975 was one of the driest on record. The 23 cm of rainfall for the four month period from May 1 to August 31 was 54 percent below the regional normal rainfall for the same period. From July 1 to August 29 there was only 5 cm of rain and 35 days in which the maximum temperature exceeded 32°C. By September, virtually all of the introductions in the 1975 nursery were dead. In contrast, almost all of the Slate plants in the border and check rows survived, possibly demonstrating superior stress tolerance. These results indicated that the evaluated intermediate wheatgrasses from Dewey's 1972 Iran collection (Dewey 1978) lacked the drought tolerance necessary for successful establishment in the central Great Plains where periodic droughts are common.

Slate is the product of several generations of selection, and increase in the Great Plains and natural selection pressure may have greatly improved the seedling vigor and stress tolerance of this cultivar. It may be possible to improve the seedling stress tolerance of the introductions from Iran by advancing them several generations under adverse conditions, allowing natural selection to occur.

The 66 cm of precipitation at the Mead Field Laboratory during the 14 month period from May 1976 to August 1977 was 60 percent below the regional normal rainfall. In contrast to the plants in the 1975 nursery, most of the plants in the 1976 nursery survived the dry establishment year, which indicated that their drought tolerance was similar to Slate's.

Mean plant width, height, forage and seed ratings, forage yield and dry matter percentages for accessions evaluated in the 1976 nursery are listed in table 1. There were significant differences among accessions for all traits (table 2). Replication effects were significant only for 1977 plant height and forage yield. Plant width and canopy height measurements made in November 1976 provided an index of seedling vigor and first year growth. Five introductions, PI 172688, PI 273732, PI 273733, PI 315355, and PI 345586 surpassed Slate in 1976 mean plant width and height (table 1). As expected, most of the accessions were inferior to Slate in forage yield. However, PI 273732, PI 273733, PI 315355, and PI 345586 had mean plant yields significantly higher than Slate, and the highest single plant yields of these four introductions greatly exceeded the yield of the highest yielding Slate plants. The minimum plant yield of PI 273732 exceeded the mean plant yield of Slate. The seed production rating of these accessions was similar to Slate.

The mean squares from the analyses of variance of plot means demonstrate that differences among accessions were the major source of variation for all traits in the nursery (table 2). The broad sense heritability estimates among accessions were very high for all traits, indicating that selection among accessions could result in considerable progress for a particular trait.

The seed used to establish the accessions in the 1976 to 1977 evaluation nursery was produced in an open-pollinated nursery and hence do not represent the original accessions but the combining ability of the original accessions with themselves and other accessions that flowered at the same time in the increase nursery. The estimates of genetic variance among accessions, $\hat{\sigma}^2_g$, are estimates of the general combining ability of the accessions.

I found significant differences among the better accessions that were harvested and analyzed on a single plant basis for all traits except for plant height in 1976 (table 3). Even among the better accessions, the among accession component of variance was the largest source of varia-

TABLE 1.—*Mean plant width, height, forage and seed ratings, forage yield and dry matter percentages for intermediate wheatgrass accessions evaluated at Mead, Nebr., 1976 and 1977*

	1976		1977								
						Forage Yield					
Accession number (PI)	Plant width	Height	Forage	Seed	Height	Mean	Relative to Slate	Range[4]	s[4]	Dry matter	Maturity
	---- *cm* ----		*production rating*[1]		*cm*	*g*	*percent*	*g*		*percent*	*rating*[2]
1. 273737	9	4	5.0	5.0	98	235	25				E
2. 172688	15	10	3.7	3.4	95	708	74	150-2000	344	46.2	S
3. 173627	6	4	5.0	4.5	110	132	14				L
4, 173630	12	6	4.0	3.6	95	692	73	100-1650	411	43.6	S
5. 204384	12	6	4.5	4.2	85	438					S
6. 204385	10	4	4.8	4.9	88	326	34				S
7. 204386	8	4	5.0	5.0	92	238	25				S
8. 204550	7	4	5.0	4.8	85	206	22				S
9. 206259	6	4	4.9	4.8	87	188	20				S
10. 206618	8	3	4.8	4.7	88	184	19				S
11. 206625	6	2	5.0	4.8	82	140	15				S
12. 208062[3]	12	4	4.3	4.4	108	393	41				S
13. 210990	8	4	5.0	5.0	94	177	19				E
14. 220497	12	7	4.3	4.0	91	450	47	75-1300	317	49.4	E
15. 220498	7	4	5.0	5.0	93	166	17				E
16. 222961	7	4	5.0	5.0	95	200	21				E
17. 223230	10	6	4.8	4.7	86	235	25				E
18. 223231	11	5	4.6	4.4	103	316	33				E
19. 229475[3]	6	3	4.9	4.9	82	148	16				S
20. 229576[3]	4	2	5.0	5.0	80	68	7				E
21. 229577	10	4	4.6	4.6	88	339	36				E
22. 229918[3]	6	2	5.0	5.0	77	87	19				E
23. 229919	10	4	4.8	4.8	96	202	21				E
24. 249145	12	6	3.8	3.7	104	530	56	200-975	217	43.7	S,L
25. 273732	21	8	2.8	3.0	104	1,547	163	275-3525	637	44.1	S
26. 273733	16	8	2.9	3.1	100	1,709	180	975-3625	587	41.8	S
27. 297872	12	4	4.9	4.9	92	191	20				E
28. 314140	8	4	4.7	4.7	84	428	45				E
29. 314189	6	4	4.9	4.9	96	144	15				E
30. 314190	7	3	5.0	5.0	96	127	13				E
31. 314191	8	4	4.8	4.7	95	241	25				E
32. 314192	8	4	4.4	4.6	107	376	40				S
33. 315067	14	6	3.1	3.1	113	1,091	115	650-1675	246	41.6	S
34. 315353	14	8	3.2	3.2	108	991	104	275-1800	409	43.2	S
35. 315355	20	8	2.8	3.0	106	1,382	145	525-2325	407	43.5	S
36. 317404	5	2	5.0	5.0	102	138	14				E
37. 325183	12	6	4.0	4.0	97	683	72	275-1300	276	49.6	S
38. 345586	18	7	3.0	3.1	110	1,622	171	275-3450	716	41.2	S
Slate	14	7	3.0	3.1	108	950	100	150-2000	344	44.5	
Mean	11	5	4.3	4.2	96	512	54			44.4	
$LSD_{(0.05)}$	3	2	0.4	0.5	11	315				4.1	

[1] 1 = Excellent; 5 = Poor.

[2] E = Early, S = Similar to Slate, L = Late.

[3] Unreplicated accessions.

[4] Range and standard deviation of individual plant yields for accessions harvested as individual plants.

TABLE 2.—*Mean squares from the analyses of variance of plot means for the accessions evaluated in the 1976-77 intermediate wheatgrass introduction nursery*[1]

Source	Degrees of freedom	Expected mean squares	Mean squares: 1976 Width	1976 Height	1977 Forage rating	1977 Seed production rating	1977 Height	1977 Yield	1977 Dry matter
			cm	*cm*			*cm*	*g*	*percent*
Replication	1	$\sigma_e + g\sigma_r^2$	9.95	0.85	0.002	0.185	790.0**	171,202.0*	0.0009
Accession	34[2]	$\sigma_e + r\sigma_g^2$	32.85**	4.92**	1.210**	1.103**	147.0**	443,980**	0.0015*
Error	34[2]	σ_e^2	2.48	0.77	0.034	0.051	30.0	24,158	0.0004
σ_g^2			15.18	2.08	0.588	0.526	58.5	209,911	0.0006
Heritability[3]			0.92	0.84	0.97	0.95	0.80	0.94	0.75

*, ** Indicates significance at the 0.05 and the 0.01 level of probability, respectively.

[1] Means of all Slate plots in replicates 1 and 2 were used as the Slate treatment means for replicate 1 and 2, respectively. Unreplicated lines PI 229475, PI 229576, PI 229918, and PI 208062 were not included in the analyses.

[2] Degrees of freedom are 33 for treatment and error for yield and 11 for treatment and error for dry matter percentage.

[3] Broad sense heritability = $\sigma_g^2/(\sigma_e^2/r + \sigma_g^2)$.

tion in the nursery for all traits. Broad sense heritability estimates among accessions was high for all traits in 1976 except plant height. The variances among plants of accessions, $\hat{\sigma}^2_w$, have a genetic, $\hat{\sigma}^2_{w_g}$, and an environmental component, $\hat{\sigma}^2_{w_e}$. This experiment was not designed to estimate $\hat{\sigma}^2_{w_g}$ because clonally propagated plants were not included in the evaluation nursery to estimate within plot environmental variation. Lebsock and Kalton (1954) used this technique with smooth bromegrass, *Bromus inermis* Leyss., and reported that plant-to-plant variation in populations produced from seed was approximately three times greater than in clonally propagated populations. Similar information is not available for intermediate wheatgrass. However, Heinrichs (1953) reported correlations for forage yield of 0.51 and 0.38 between clonal means of intermediate wheatgrass plants derived from PI 98568 and the means of their polycross and open-pollinated progenies, respectively, indicating that a substantial proportion of the variation for forage yield was genetic. Assuming that only one-third of the variance within accessions is genetic, the ratio $\sqrt{1/3 \cdot \hat{\sigma}^2_w} / \overline{X}$ is 0.18 for first-year plant width and height and 0.21 for second-year forage yield, indicating that there may be considerable genetic variation within the better accessions. The standard deviation among plants of a particular accession for forage yield ranged from 22

TABLE 3.—*Mean squares from the analyses of variance of accessions harvested for forage yield on an individual plant basis in 1977*

Source	Degrees of freedom	Expected mean squares	Mean squares: 1976 Width	1976 Height	1977 Forage rating	1977 Seed production rating	1977 Height	1977 Yield
			cm	*cm*			*cm*	*g*
Replication	1	$\sigma_w^2 + w\sigma_e^2 + gw\sigma_r^2$	3.40	5.78	0.153	0.253	64.80	4,924,041*
Accession	9	$\sigma_w^2 + w\sigma_e^2 + rw\sigma_g^2$	332.78**	18.67	6.188**	3.702**	1,072.11**	5,532,047**
Error	9	$\sigma_w^2 + w\sigma_e^2$	51.30	15.01	0.882	0.052	56.54	920,512
Within accession error	300	σ_w^2	23.57	4.82	0.342	0.208	91.84	170,250
$LSD^1_{(0.05)}$			4.0		0.53	0.13	4.25	543
σ_g^2			8.79	0.11	0.166	0.114	31.73	144,110
Heritability[2]			0.84	0.20	0.857	0.99	0.95	0.83

*, ** Indicates significance at the 0.05 and the 0.01 levels of probability, respectively.

[1] LSD value listed only where the F value was significant.

[2] Broad sense heritability = $\sigma_g^2/(\sigma_w^2/rw + \sigma_e^2/r + \sigma_g^2)$.

TABLE 4.—*Correlation of various traits with forage yield in accessions of intermediate wheatgrass at Mead, Nebr., 1977*

Trait and year measured	Correlation coefficient
Plant width (1976)	0.85**
Canopy height (1976)	0.74**
Forage rating (1977)[1]	0.92**
Seed rating (1977)[1]	0.88**
Dry matter, percent (1977)	−0.50**

** Indicates significance at the 0.01 level of probability.

[1] Algebraic signs of these correlations were changed from negative to positive since low ratings indicated superiority.

TABLE 5.—*Expected variances of a treatment mean ($s^2_{\bar{x}}$) and the resulting LSD.$_{.05}$ values for forage yield when the number of replications and plants within a replication are varied*

Number						
Replications	Plants within plots	Error degrees of freedom (t-1)(r-1)[1]	σ^2_w/rw [2]	σ^2_e/r [2]	$s^2_{\bar{x}}$	LSD.$_{.05}$
n	*n*					*g*
1	40	not estimatable				
2	20	9	4,256	23,445	27,701	532
4	10	27	4,256	11,723	15,979	367
5	8	36	4,256	9,378	13,634	334
10	4	81	4,256	4,689	8,945	266

[1] The number of treatment (t) = 10.

[2] $\sigma^2_w = 170250$ and $\sigma^2_e = 46891$ (table 3).

to 70 percent of the mean forage yield of an accession, which also indicates that there is considerable variation for forage yield within accessions (table 1).

Seedling vigor, as indicated by first-year plant width and height, was positively correlated to second-year forage yield (table 4), and seed production rating was positively correlated with forage yield. Henrichs and others (1962) and Lawrence (1962) have reported high positive genetic and phenotypic correlations, respectively, between forage and seed yield of intermediate wheatgrass, indicating that it should be possible to improve both traits simultaneously by breeding.

Several methods can be used to evaluate intermediate wheatgrass introductions. Replicated space-planted nurseries in which adapted cultivars are included provide one method for economical and effective screening of the accessions' mean performance. If clonally propagated lines are included in the evaluation nursery, estimates can be obtained of the genetic variation within accessions. Only a limited number of plants of each accession can be evaluated. Smaller experimental errors and consequently better discrimination among accessions can be obtained using four or more replications and 10 or fewer plants per replication than by having fewer and larger evaluation plots (table 5).

Although I evaluated only a small number of the total available intermediate wheatgrass introductions, I found considerable variation among accessions that breeders should be able to use. The accessions that outyielded the adapted and productive check cultivar should be of particular value to breeders and can possibly be rapidly developed into cultivars. Considerable variation within accessions was observed, which should be useable for breeding methods such as restricted recurrent phenotypic selection (Burton 1974). The surprisingly large number of superior introductions in the limited number of accessions evaluated to date suggest that the continued reliance on cultivars developed from a single introduction appears unwarranted.

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